REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
19-12-2005	Article	2001-2003; 2004

4. TITLE AND SUBTITLE
Cerebral blood flow changes: rat studies in rheoencephalography.

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
6. Bodo M, Frederick J. Pearce FJ, Armonda RA.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Walter Reed Army Institute of Research
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503 Robert Grant Avenue
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES
Physiol Meas, 2004 25 1371-1384

14. ABSTRACT
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15. SUBJECT TERMS
Cerebrovascular reactivity, CBF, REG, LDF, carotid flow, rat

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Michael Bodo
uu	uu	uu	uu	14	19b. TELEPHONE NUMBER *(Include area code)* 301 319 9880

INSTITUTE OF PHYSICS PUBLISHING PHYSIOLOGICAL MEASUREMENT

Physiol. Meas. **25** (2004) 1371–1384 PII: S0967-3334(04)77451-1

Cerebrovascular reactivity: rat studies in rheoencephalography

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Received 8 March 2004, accepted for publication 9 August 2004
Published 7 September 2004
Online at stacks.iop.org/PM/25/1371
doi:10.1088/0967-3334/25/6/003

Abstract

Here we describe a correlative study of cerebral blood flow (CBF) using global, local CBF and carotid flow measurements. The primary objective of this study was to establish a relationship between REG and CBF autoregulation. Rheoencephalography (REG), a rarely used method to measure CBF, is a potential tool of non-invasive continuous life sign monitoring and detection of early cerebrovascular alteration. However, the anatomical background of REG is not clearly understood. Two experimental studies were undertaken on anesthetized rats to define two CBF measurements: (1) CO_2 inhalation, and, (2) clamping of common carotid arteries. Measurement of CBF was taken with REG, laser Doppler flowmetry (LDF) and carotid flow by Doppler ultrasound. Data were off-line processed. During CO_2 inhalation, the increases in REG and LDF were significant ($p = 0.0001$), while carotid flow and systemic arterial pressure decreased. During carotid artery clamping, the decrease in REG and Doppler ultrasound was significant ($p = 0.0001$). REG showed cerebrovascular reactivity, indicating the relationship to arteriolar changes. Compared to LDF and carotid flow, only REG showed the classical CBF autoregulation.

Keywords: cerebrovascular reactivity, CBF, REG, LDF, carotid flow, rat

1. Introduction

Monitoring cerebral blood flow (CBF) has vital importance both during clinical (neurosurgical) postoperative care and for life sign detection. Ideally, continuous and non-invasive monitoring

of CBF would be performed. Cerebrovascular reactivity used to be measured to establish the status of CBF autoregulation, and to evaluate patient status (Aaslid 2002). The goal of CBF autoregulation is to maintain adequate perfusion pressure to the brain (Strandgaard and Paulson 1984). In recent clinical practice Doppler ultrasound is used for CBF monitoring. However, Doppler measurement takes several minutes to perform and must be repeated several times daily. The traditional Doppler probes are not designed for continuous monitoring. Flat probes are available but difficult to maintain for continuous monitoring. The holding frame for conventional Doppler probes is inconvenient for continuous monitoring. In non-clinical application, such as physiological status monitoring for military combat casualty care, and air and space research, neither Doppler technique is adequate.

Rheoencephalography (REG; pulsatile electrical impedance measured on the head) is an accepted (Anonymous 1997) but rarely used CBF method. Even though understanding of the anatomical background is unclear, REG is a potential tool for non-invasive continuous life sign monitoring. Several correlative studies established a correlation between (1) electrical impedance versus flow/volume and pressure (Nyboer 1960, Geddes and Baker 1989, Shender and Dubin 1994), and (2) CBF and REG (Jacquy *et al* 1974, Hadjiev 1968). However, no data are known describing the relationship between REG, local CBF and carotid flow. To evaluate REG we pointed out two known tests of CBF manipulation: CO_2 inhalation to increase CBF (Hurn and Traystman 2002) and ligature of common carotid arteries to decrease CBF (Eklof and Siesjo 1973). In order to avoid potential extracranial interference, we used intracranial and invasive derivations. Here we report results of comparing global CBF (REG) with local (cortical) CBF (laser Doppler flow—LDF) and carotid flow (Doppler ultrasound) on rats.

2. Methods

The work described here was conducted under a protocol approved by the Institute's Laboratory Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals (Anonymous 1966), in facilities that are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

Male Sprague Dawley rats (230–500 g) were anesthetized with pentobarbital (50 mg kg, i.p.). Body temperature was maintained at 37 °C with a heating pad-rectal thermometer closed-loop system (Harvard Apparatus, Edenbridge, KT). A tracheotomy was performed by inserting a polyethylene tube into the trachea. Both common carotid arteries were exposed (Anonymous 1996). A silk suture snare was placed under each common carotid artery in order to help the placement of flow probes and for use as a ligature (Mima 1995). The femoral arterial cannula was inserted for monitoring SAP. The rat was placed in a ventral recumbent position with the head in a stereotaxic frame. The skin was incised over the sutura sagittalis (30 mm in length), and the skull was exposed between the sutura coronalis and the s. lambdoidea. The dermis, subdermal layers, periosteum and muscle over the parasagittal regions were removed; the bone was rinsed with hydrogen peroxide to prepare the surface for adhesivity to dental acrylic applied to hold the REG electrodes. Two stainless steel intracranial electrodes (Plastics One, Roanoke, VA) with 5 mm long, uninsulated surfaces were inserted into the brain via holes drilled with a 1 mm dental drill and were fixed with instant adhesive (Loctite, Rocky Hill, CT) and cranioplastic acrylic cement (Plastics One, Roanoke, VA). The localization of the electrodes was as follows: 3 mm lateral to the sutura sagittalis; 3 mm anterior to the coronal suture; 3 mm anterior to the lambdoid suture. These electrodes were used to measure global CBF in intrahemispherical (fronto–occipital) derivation. The balanced resistance was in the range of 7–10 Ω. A 6.5 mm diameter hole was drilled into the parietal

bone (equal distance between lambdoid and coronal suture) for local CBF measurement with LDF. The probe was attached to a micromanipulator and placed on the dura mater; the gap was filled with physiological saline.

The total number of rats measured by REG during CO_2 administration was $n = 11$, in trials = 63. Subgroups were formed for REG and LDF or REG and carotid flow measurement. REG groups A and B indicate different rats where REG only was measured; in REG/LDF, these two modalities were measured together. It was not possible to measure all three modalities in one session simultaneously, therefore paired subgroups were formed. Carotid ultrasound was measured while the rat was in the supine position; during LDF measurement the rat was in a sphinx position in a stereotaxic frame. Ten per cent CO_2 gas (Nova Biomedical, Waltham, MA) inhalation was used to increase CBF during 1 s. The gas was administered by means of a specially developed four-way adapter, which served as an interface between the tracheal cannula, gas inflow tube and gas monitor sampling tube, so that the inhaled CO_2 gas was always mixed with room air and therefore never reached the named source concentration level.

Occlusion of the carotid arteries was accomplished by aneurysm clips or carotid ligatures, while the rat was in a supine position ($n = 5$, 13 trials). Readings from the Doppler flow probes verified the lack of blood flow through the carotid arteries.

Measurement and data acquisition were performed using the following equipment: *In vitro*, for integral validation; function generator (Agilent, 33120A, Hewlett Packard, Palo Alto, CA), signal: 5 Hz, 100–1000 mV PP, sine wave; AD sampling rate: 360 Hz; PC: an IBM compatible PC was used for data collection and processing with Advantech (Sunnyvale, CA) PCL-718 type A/D card (12 bit resolution). Datalyser (WRAIR, Baranyi), Dadisp (DSP Development Corporation, Newton, MA) and Excel (Microsoft, Redmond, WA) were used for data collection and processing.

In vivo: REG by impedance amplifier (with 46 kHz measuring frequency—KR-Ea RHEO Preamp, Galileo, Italy); local CBF (LDF) by integrating probe, (6 mm diameter, seven sensors) and Periflux System 4001 (Perimed, Sweden); carotid flow using aquasonic ultrasound gel (Parker Laboratories, Fairfield, NJ), and by microcirculation probes (0.5 V and 0.7 V), T201 ultrasonic bloodflow meter (Transonic Systems, Ithaca, NY); electrocardiogram (EKG, Lead II) by ECG/Biotach amplifier (Gould Electronics, Cleveland, OH); SAP by transducer (Maxxim Medical, Athens, TX) and Digi-Med blood pressure analyzer (Micro-Med, Louisville, KY); signal preconditioning for the A/D conversion by dc and universal amplifiers (Gould Electronics, Valley View OH); and CO_2 concentration by CD-3A carbon dioxide analyzer and R-2 flow control (Ametek, Pittsburgh, PA).

The physiological signals were digitized (630 Hz) and visualized by REDIREC software (Heilig *et al* 1998). Data processing was performed offline. Calculation was accomplished as follows: two 5 s segments were used per trial, one to establish a baseline and another during challenge (maximal amplitude in the case of CO_2 inhalation and minimal amplitude during clamping). REG pulse integral (REG group A) and amplitude and integral (area below the curve; REG group B) values were measured by DataLyser using LabWindows (National Instruments, Austin, TX) modules. Digital filtering was used (3–100 Hz, Chebysev) to isolate the useful REG signal, that is, to eliminate the respiratory subharmonic. The first step of the calculation was to establish a difference between the baseline and challenge, which was expressed as a percentage of the baseline. Afterwards the individual results were averaged: in this way, the differences in amplification were elucidated, allowing for creation of a group average. Excel software was used for data collection, and Minitab (Minitab Inc, State College, PA) for statistics. REG integral and amplitude values are shown in arbitrary units (obtained from the AD converter), and are presented here as mean $\pm$ SD. Probability <0.05 was

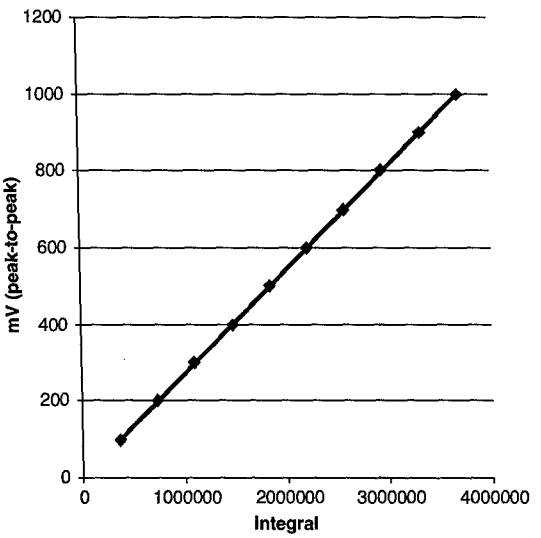


Figure 1. Calibration of voltage–integral correlation. The signal was a 5 Hz sine wave, amplitude range 100–1000 mV. The time window of integration was 5 s. The equation of the integral (y)/input voltage (x) was $y = 0.0003x$. correlation coefficient (R^2) 1. For other details see section 2.

considered statistically significant. Other statistical characteristics were as follows: Pearson's correlation coefficient indicates the strength of a linear relationship, and Spearman's rank correlation indicates the strength of a monotonic relationship; the p-values indicate how likely the coefficients are equal to zero (i.e. the null hypothesis tested is that each coefficient is equal to zero).

3. Results

3.1. In vitro: integral validation

Amplitude has a linear relationship and tight correlation to the integral; correlation coefficient = 1 (figure 1). Amplitude change can be measured by integral.

3.1.1. In vivo: (1) CO_2 inhalation. The typical reaction was as follows: after CO_2 inhalation, REG (amplitude and integral) increased, then returned to the baseline. At first, LDF began to decline but then increased. SAP decreased. Pulse amplitude of carotid flow initially decreased, first in correlation with SAP decline and later often showed reactive hyperemia (figure 2).

In all CO_2 inhalation groups, REG amplitude and integral increased significantly; p ranged from 0.0048 to 0.0001 (tables 1 and 2).

In the REG/LDF group, both REG and LDF amplitude and integral increased significantly $p < 0.0001$ (table 2). The correlation of REG and LDF change was significant, with a p-value less than 0.01 (table 3).

3.1.2. In vivo: (2) Carotid occlusion. During carotid artery clamping, REG amplitude and integral significantly decreased, $p < 0.0001$ (figure 3 and table 2).

Table 1. REG integral changes during CO_2 inhalation (REG group A).

Rat ID	BL	MAX	Increase (%)
158-1	0.071	0.196	176.06
158-2	0.097	0.278	186.60
158-3	0.109	0.341	212.84
158-4	0.1	0.324	224.00
157-1	0.079	0.586	641.77
157-2	0.133	0.454	241.35
157-3	0.141	0.508	260.28
157-4	0.112	0.383	241.96
7-2-1	0.061	0.221	262.30
7-2-2	0.012	0.031	158.33
7-2-3	0.639	2.491	289.83
8-1-1	0.04	0.066	65.00
8-6-1	0.101	1.562	1446.53
8-6-2	0.191	2.311	1109.95
8-6-3	0.01	0.072	620.00
8-6-4	0.01	0.063	530.00
8-6-5	0.0110	0.059	436.36

Rat ID: identification of a rat/trial; BL: value of 5 s baseline REG integral; MAX: value of 5 s integral during maximal REG amplitude; increase: value expressed as percentage of BL. The group average increase was 417.83 ± 366.74. Total number of rats was 5, total number of trials was 17. The increase was expressed as percentage of BL; it was significant ($p < 0.0048$). The Pearson's correlation coefficient was 0.79, and the Spearman correlation coefficient was 0.88. The p-value associated with each correlation was less than 0.0001. For other details see section 2.

Table 2. Rheoencephalogram (REG) and laser Doppler flow (LDF) changes during CO_2 inhalation and carotid clamping.

	Baseline	CO_2 inhalation	Probability	Increase (%)
REG group B				
REG amplitude	0.023 ± 0.0412	0.101 ± 0.1285	0.0007	66.92 ± 21.81
REG integral	0.441 ± 0.6246	1.25 ± 1.6159	0.0005	214.28 ± 180.55
REG/LDF group				
REG amplitude	0.009 ± 0.0049	0.030 ± 0.0314	0.0001	68.78 ± 14.56
REG integral	0.056 ± 0.0711	0.174 ± 0.1867	0.0001	243.76 ± 138.91
LDF amplitude	135.68 ± 91.25	849.3 ± 740.3	0.0001	78.07 ± 11.40
LDF integral	0.056 ± 0.0711	0.174 ± 0.1867	0.0001	307.96 ± 304.69
	Baseline	Clamping	Probability	Decrease (%)
REG amplitude	74.58 ± 41.84	22.61 ± 8.67	0.0001	61.55 ± 20.06
REG integral	0.0500 ± 0.0169	0.0122 ± 0.0057	0.0001	27.53 ± 18.03

Amplitude and integral values are presented here in arbitrary units, as mean ± SD. Inter-group values may differ due to different gain settings of REG amplifier. The difference, for example, in the case of 0.023 and 0.101, 66.92% is not the ratio of these numbers but is rather the average difference of the whole group, which was first calculated individually. Because of unknown differences in individual amplification among rats between each measurement, such calculations would be misleading. Sample size: REG group B, 5 rats, 25 trials; REG/LDF group: 4 rats, 11 trials; carotid clamping: 5 rats, 13 trials; BL: baseline. For other details see section 2.

The purpose of showing tables 1, 2 and 3 is to present REG data introducing the results from various points of view as follows: (1) within one group (REG group A, table 1);

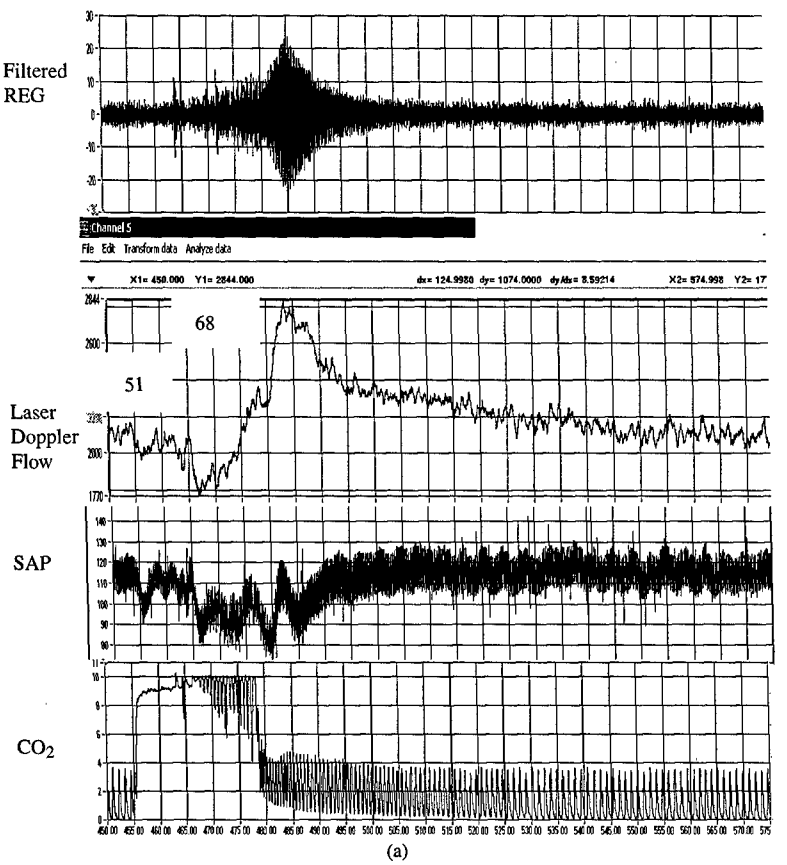
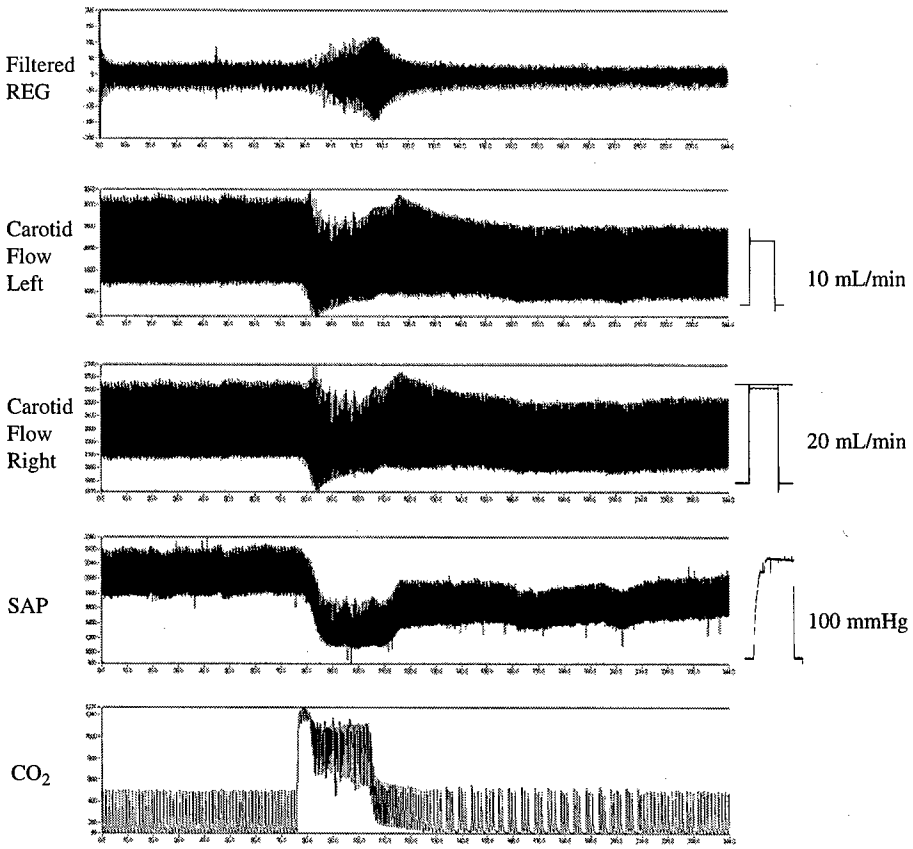


Figure 2. Typical effect of CO_2 inhalation on rheoencephalogram (REG) and laser Doppler flow (LDF) (a) and on REG and carotid flow (b). (a) The CO_2 increase indicates the start of the 10% CO_2 gas administration. Immediately after the gas administration, systemic arterial pressure (SAP) decreased from 120/110 mm Hg to minimum 90/80 mm Hg, followed first by the LDF decrease, than by an increase in LDF. In contrast, REG increased during the whole period. The LDF and REG maximums were identical in time. The REG recovery was shorter than that of the LDF. The LDF baseline was 51, and the maximum was 68 (flux, perfusion units). The rat/trial ID was 8/6/02-2; REG was an intrahemispherical (right-side) derivation. The time window was 125 s. (b) Immediately after the 10% CO_2 gas administration SAP decreased from 160/100 to 100/40 mm Hg, followed by a carotid flow decrease, and later by a slight increase. Left carotid baseline was 10.9/1.2 mL min^{-1} (minimum: 8.14/-2.5). Right carotid baseline was 6.0/ 0.5 mL min^{-1} (minimum: 4.8/-2.0). For better visibility the recording traces were blown up; the real flow calibration values appear on the right side. Amplification of left and right carotid flow differed. REG showed a gradual increase during SAP decrease and later a decrease. In this case the carotid flow decrease was moderate, compared to others. The rat/trial ID was 158/4. REG was an intrahemispherical (left-side) derivation. The time window was 122 s. The calibrations were as follows: carotid left, 10 mL min^{-1} ; carotid right, 20 mL min^{-1} ; SAP: 100 mmHg. *Both Panels*: filtered REG: REG signal was filtered with 3–100 Hz; CO_2: gas concentration in the tracheal cannula.

(2) present REG and LDF data (REG/LDF group, table 2); and (3) present the correlation between REG and LDF (table 3).



(b)

Figure 2. (Continued.)

Table 3. Statistical summary of the CO_2 inhalation results in REG/LDF group.

Rat ID	Number of time points	Pearson's (r)	Spearman's (r)
7/11/02	29	0.63	0.73
7/18/02	29	0.80	0.74
7/23/02	35	0.71	0.66
7/25/02	24	0.63	0.81
Total	117	2.77	2.94
Mean	29.25	0.69	0.74
SD	4.50	0.08	0.06

The 10 s time window of analyzed sample was characterized by three or four values. Pearson's (r) for all 117 time points = 0.80. Spearman's (r) for all 117 time points = 0.73.All correlations are statistically different from zero with a p-value less than 0.01. Rat ID: identification of a rat. For other details see section 2.

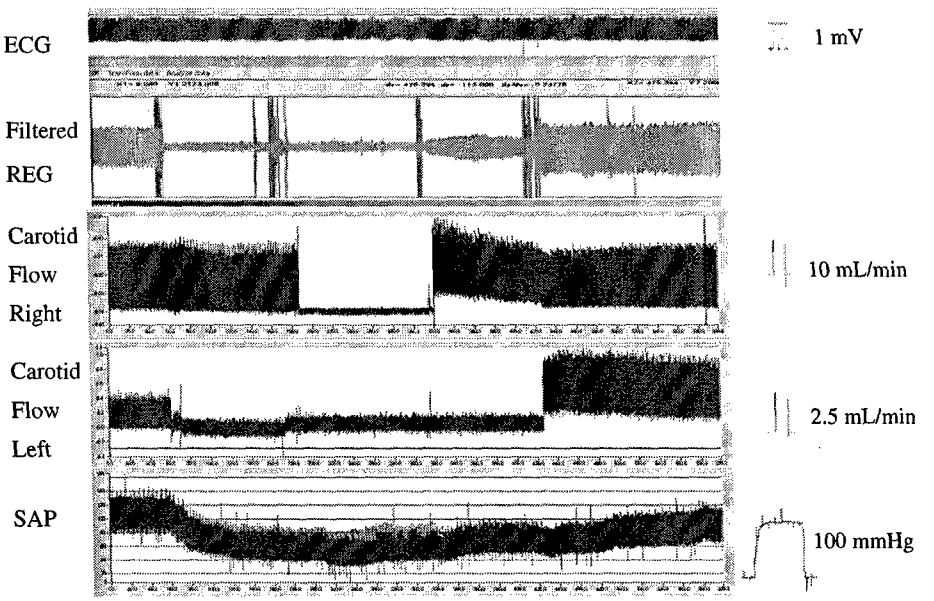


Figure 3. Effect of clamping of common carotid arteries. Following the first clip placement on the left carotid artery, the rheoencephalogram (REG) amplitude (filtered REG) decreased; the placement of the second clip right carotid artery had no further decrease. During the clamping period there was no pulse amplitude observed in either carotid trace. For better visibility the recording traces were blown up; the real flow calibration values appear on the right side. Amplification of left and right carotid flow differed. A few minutes after removal of one clip from the right carotid artery, REG amplitude moderately increased, and after removal of the second clip from the left carotid artery, the REG amplitude returned to slightly above the baseline level. Similarly, both carotids showed a slight hyperemic reaction. The baseline systemic arterial pressure (SAP) value was 135/80 mm Hg; during clamping, the minimal value was 80/40 mm Hg. The rat/trial ID was 5-23-02/11. REG was an intrahemispherical (left-side) derivation. The time window was 10 min; REG filter was 3–100 Hz. ECG: electrocardiogram.

(This figure is in colour only in the electronic version)

4. Discussion and conclusion

The primary objective of this study was to establish a relationship between REG and CBF autoregulation. REG (amplitude and integral) increased during CO_2 inhalation, but decreased during carotid artery clamping. During CO_2 inhalation carotid flow and SAP decreased; REG and LDF changes were partially correlated. The LDF, REG and carotid flow each showed a different aspect of the CO_2 reaction since each represents a different level of CBF. The classical CBF autoregulation was observed within the REG signal only. During carotid clamping REG reflected the decreased CBF. Using REG, we performed several hundred CO_2 inhalations on rats. We reported only some of our results in this paper. The observed REG pulse amplitude changes were statistically significant and practically relevant. The goal of this study was not to perform basic scientific research or to analyze the origin of REG but rather to compare REG to other CBF modalities and CBF autoregulation in order to allow us to build a life sign monitor using REG.

4.1. CBF

The brain has ongoing, substantial energy requirements but minimal stores of energy-generating substrates. As a result, it is completely dependent on a continuous, uninterrupted supply of substrate (oxygen, glucose). Although the demand by the brain for energy-generating substrates is substantial (the central nervous system consumes 20% of the oxygen (that is, 170 mmol/100 g per min or 3–5 ml O_2/100 g brain tissue per mm or, approximately, 40–70 ml O_2 min) and 25% of the glucose (31 mmol/100 g per mm) utilized by the resting individual under physiological conditions), this is met more than adequately by the 15% of the resting cardiac output (750 ml mm) which perfuses the brain (mean global CBF = 50 ml/100 g brain tissue per mm; range 45–55 ml/100 g per mm) approximately 80% to grey matter and 20% to white matter. Indeed, normally, the supply of oxygen (approximately 150 ml O_2 min^{-1}) is considerably in excess of requirements (around 40–70 ml O_2 min^{-1}) such that the brain extracts only 25–30% of that supplied. In addition, the brain can conserve energy and, hence, decrease demand by switching off many of its metabolic processes before its reserves have been compromised when the delivery of substrate reaches 'critical' values. However, the flip side of this argument is that, paradoxically, the brain cannot tolerate significant increases in the volume of the contents of the rigid container in which it is enclosed. Moreover, because the brain's own store of energy-generating substances (glycogen, glucose, oxygen) is small (so small that at normal rates of adenosine phosphate production, the stores of glycogen in the brain would be exhausted in less than 3 min) it is uniquely dependent on a continuing, and adequate, supply of substrate (Fitch 1999).

4.2. CBF autoregulation

Autoregulation in the cerebral circulation may be defined more pragmatically as the mechanism that protects the brain against the dangers of hypoxia at low perfusion pressures and against the risks of brain edema at high arterial pressures. Based on this definition, cerebral autoregulation may be thought of as a homeostatic mechanism that is superimposed over and above the baroreceptor reflexes. The baroreceptors, strategically located at the most proximal locations in the cerebral circulation, provide the first line of defense against acute ranges in arterial pressure. Autoregulation then serves as the next line of defense by helping to maintain constant cerebral capillary pressure, thus assuring a steady supply of essential metabolites and simultaneously protecting the blood–brain barrier. Several hypotheses (myogenic, neurogenic and metabolic) have been proposed to account for the mechanisms that underlie autoregulation, detailed elsewhere (Chillon and Baumbach 2002). The anatomical background of CBF autoregulation is the arteriole. The arterioles are the last small branches of the arterial system, and they act as control valves through which blood is released into the capillaries. The arteriole has a strong muscular wall that is capable of closing the arteriole completely or of allowing it to be dilated several fold, thus having the capability of vastly altering blood flow to the capillaries in response to the needs of tissue (Ganong 2001, Kontos *et al* 1978). This arteriolar functioning is visualized by functional MRI in brain imaging (Vainrub *et al* 2004).

The classical CBF autoregulation means that CBF has a limited autonomy (between systemic arterial pressure about 50–150 mmHg). In other words, the cerebrovascular reactivity works 'against' the SAP within this range. In the presented experiments this behavior was shown clearly only by REG. The carotid flow and the cortical flow had a mixed or dual response, i.e. at the beginning of CO_2 inhalation they followed SAP, which is not an autoregulatory response, and in the later phase they often showed a reactive hyperemia. The classical definition of CBF autoregulation does not describe these two phases (Strandgaard and Paulson 1984, Chillon and Baumbach 2002). In our measurements during CO_2 inhalation, systemic

arterial pressure always decreased (see figure 2). Consequently, the cerebrovascular reaction involves CBF and volume increases. This is why we used the term autoregulation. We used the term autoregulation as a synonym, covering any cerebrovascular reaction including CO_2 or changes in systemic arterial pressure, since they are interrelated.

4.3. CBF and CO_2

In man, 5% and 7% CO_2 inhalation raises CBF by approximately 50 and 100%, respectively (Kety and Schmidt 1948). Cerebral vasodilatory responses to hypercapnia and hypoxia are consistent, reproducible and reversible. Accordingly, changes in systemic gas tensions have been frequently employed as a test of essential cerebrovascular reactivity under normal and pathophysiologic conditions. The mathematical expressions that govern the relationship between CBF and partial pressure of carbon dioxide have been described (Reivich 1964, Olesen *et al* 1971). In these experiments we demonstrated that REG detects CBF (and/or volume) increase during CO_2 inhalation, similar to other quantitative CBF techniques.

4.4. CBF monitoring

The detection of pathophysiological events in the brain and circulation by continuous, non-invasive physiological monitoring following brain injury and/or operations has the potential to improve survivability of patients. The primary aim of managing patients with acute brain injury in the intensive care unit is to minimize secondary injury by maintaining cerebral perfusion and oxygenation. The mechanisms of secondary injury are frequently triggered by secondary insults, which may be subtle and remain undetected by the usual systemic physiological monitoring. Continuous monitoring of the central nervous system in the intensive care unit can serve two functions. Firstly it will help early detection of these secondary cerebral insults so that appropriate interventions can be instituted. Secondly, it can help to monitor therapeutic interventions and provide online feedback (Gupta 2002).

One potential monitoring method can be the REG, because of its non-invasiveness, good time resolution and because of showing cerebrovascular reactivity and/or CBF autoregulation. The organ of CBF autoregulation is the arteriolar portion of the circulatory system (Chillon and Baumbach 2002, Guyton 1991). However, in clinical practice the carotid and middle cerebral arteries are used to determine CBF autoregulation. These facts can explain part of related contradictory findings: various parts of arterial system have different involvement of CBF autoregulation (Kontos *et al* 1978).

There are two related background problems of CBF monitoring. (1) Technical: there can be contradiction between techniques, showing local or global CBF, or carotid flow. In our study we overcome this problem, measuring simultaneously these signals and describing the diversity of these methods. Even, the applied LDF integrating probe (covering much larger area than the usual single fiber probes) was unable to show the classical CBF autoregulation: the signal was influenced by SAP change. (2) Biological: the essential nature of CBF is the heterogeneity during both physiological and pathological conditions. This is why the global and local flow data can show divergent direction of their changes. Further complication can be, that what is true within physiological range of CBF regulation can be false during pathological CBF state, such as increased intracranial pressure (ICP) or hypotension. This means that during edema formation (ICP increase, CBF decrease) REG can reflect not the decreased cerebral perfusion pressure but the increased water content of the cranial cavity (McHenry 1965, Bodo *et al* 1986). For monitoring purpose the necessity of using the combination of signals seems to be promising as was proposed by Klaessens *et al* (2003) in order to elucidate such misleading information.

The test of cerebrovascular reactivity in clinical practice is a routine test to evaluate the status of a patient during neurosurgery postoperative care: nonreactive cerebrovascular vessels are a bad prognosis. The only way of continuous monitoring by Doppler technique is the use of a special frame holding the Doppler probe, which is often impossible even in the neurosurgery postoperative care. In the other fields (aviation, and space flight, for military and emergency medicine) it is difficult to imagine that such a frame would be realistic to apply for continuous CBF monitoring. The available flat Doppler probes can be used without a frame, but keeping them in place is a problem, and it is far from ideal for the targeted applications. REG has potential as a convenient, noninvasive continuous monitoring tool, better than the currently used Doppler monitoring.

4.5. REG

The United States FDA definition (Anonymous 1997) states: Sec. 882.1825. 'Rheoencephalograph: (a) Identification. A rheoencephalograph is a device used to estimate a patient's cerebral circulation (blood flow in the brain) by electrical impedance methods with direct electrical connections to the scalp or neck area'. In other words, the FDA definition includes the word 'flow'; hence, our use of the term in our text. On the basis of previous and recent data (Nyboer 1960, Bodo *et al* 2001, 2003), REG is actually a reflection of volume rather than flow. This statement is supported by human and animal measurements we have previously conducted (Bodo *et al* 1986). However, a technical problem that should be mentioned here is that the REG device that we used for most measurements (except in Rat/1 group) works only with bipolar derivation and is able to measure only ac (pulsatile impedance). Basic impedance is compensated at the beginning of the measurement in this type of impedance amplifier, which is not able to record the dc component as a signal. The tetrapolar devices are able to record basic impedance and consequently to detect absolute volume changes, as they are used in clinical practice for venous outflow phlebography and impedance cardiography.

REG pulse amplitude increased as SAP decreased because of arteriolar vasodilation and increased blood volume within the skull. The increased blood volume is demonstrated by increased REG pulse amplitude, due to the fact that 'blood and even more cerebrospinal fluid are better conductors for alteration current than brain tissue' (Nyboer 1960, Jenkner 1986). REG showed the classical CBF autoregulation, indicating its close relationship to arteriolar changes. Early CBF–REG studies did not focus on this topic (Hadjiev 1968, Jacquy *et al* 1974, Moskalenko 1980, Jenkner 1986).

REG and CBF correlation has been described earlier (Hadjiev 1968, Jacquy *et al* 1974, Jenkner 1986). However, the correlation of global, local CBF and carotid flow was not investigated. This is the basic issue in selecting any of these CBF methods for lifesaving monitoring purpose. Our previous and recent results agree with those of others for REG (or electrical impedance) and CBF (volume, flow or pressure) correlation (Nyboer 1960, Bodo *et al* 2001, 2003). The new conclusion that can be drawn from the results of the present study is that REG reflects cerebrovascular reactivity, in other words, the classical CBF autoregulation, since SAP decreased during CO_2 inhalation and increased during aortic compression and hypotension (Bodo and Pearce 2004, Bodo *et al* 2004).

4.6. REG measurement

The usual method used to quantify a REG signal is similar to that of other pulse wave measurements—amplitude (minimum maximum distance), its first derivative and integral measurement (Geddes and Baker 1989). Both variables detected the applied CBF

manipulations. The application of the REG derivative and integral has an advantage using computer data processing (Bodo *et al* 1995, Bartocci *et al* 1999). The signal amplitude–integral correlation confirmed the applicability of integral calculation. 'Integral' refers to the area under the curve. No related data have been previously found or published. We performed an *in vitro* validation, comparing (sign wave) amplitude to its integral with the same data processing technique used for REG measurements on rat data. The relationship was a clear linear correlation (see figure 1). The reason why the use of an integral is a practical way to measure pulse waves is that the 5 s time period contains 35–40 REG pulses in rat. To measure these pulses individually takes 30–40 cursor (manual) measurements, but using the integral calculation takes only one measurement in Datalyser software. We used both methods to compare the integral and the REG pulse amplitude on the same data. The *in vivo* REG data confirmed the *in vitro* amplitude–integral correlation.

4.7. Anesthesia

It is known that the anesthesia influences the physiological reactions, CBF level and cerebrovascular reactivity (Roberts 2000). We can hypothesize that the effect of used pentobarbital was equal for all three CBF modalities. The observed CO_2 reaction on REG showed a small amount of variability. If a rat showed signs of awakening, a further small amount of anesthetic agent was introduced i.p. Therefore, we can be reasonably certain that reported differences in REG reaction are due to differences in the depth of anesthesia.

4.8. Conclusions

The main novelty of this paper is to demonstrate the difference among the mentioned CBF modalities and their different involvement in CBF autoregulation. Neither Jenkner nor Moskalenko discusses REG as we do, since they have no such correlative modalities (SAP, REG, local CBF, carotid flow, exhaled CO_2), as we did. These studies confirmed that REG qualitatively reflects changes in CBF and/or cerebral blood volume during known CBF manipulations. Therefore, computerized REG monitoring may be a potentially viable non-invasive and continuous method for assessing changes in CBF. The early change in cerebrovascular reactivity offers the use of REG for non-invasive bedside monitoring and stroke prevention promising (Bodo *et al* 1995). Understanding of both physiologic and pathologic responses, measured by REG, is an important first step in this process. Correlating patterns of REG to various pathologic states could establish the role of REG in life sign monitoring. Clinical applications can include monitoring patients at risk for stroke due to trauma, vasospasm of subarachnoid hemorrhage and tromboembolic stroke. Also, further studies are needed in order to test the CBF autoregulation with graded increases and decreases in SAP and ICP (McHenry 1965, Moskalenko 1980). Since the intracranial origin of the REG has a discussion (Jenkner 1986, Perez *et al* 2000, Basano *et al* 2001) as a next step, we have to study the correlation of intracranial–extracranial signals in animal experiments. Further studies involve the use of EEG and REG in establishing the sequence of disappearance of vital sings during hemorrhage.

Acknowledgments

The authors thank Anderson W, Arora N, Baranyi L, Cappello S, Fleming E, Hills S, Morrissette C, Rose T and Stueber M for their help, and Janice Meer for her assistance with the manuscript. This work was performed while the first author held a National Research

Council (WRAIR Department of Resuscitative Medicine) Research Associateship. Funding for the research was granted by Walter Reed Army Institute of Research (WRAIR), Department of Resuscitative Medicine.

There were no conflicts of interest related to this study. The material reflects the views of the authors and should not be construed to represent those of the Department of the Army or the Department of Defense.

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